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REPORT ON THE YEAST-LIKE ORGANISMS ASSOCIATED WITH BLUE STAIN OF WOOD

By

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Introduction

On September 26, 1931, twelve agar slant cultures from Blue Stain infected wood, which had been infested with insects, were obtained from Dr. C. Rumbold, Associate Pathologist in Forest Pathology, at Madison, Wisconsin. Experimental work was begun to isolate the yeast-like organisms always appearing along with the Blue Stain fungi, with the intention later to identify them and determine something of their physiological actions.

The methods of isolating these organisms, and the data on their morphologic, cultural and physiological characters are given in the following report. These latter data have been compiled in Tables I and II. Two photographs of typical cultural reactions are also given.

Experimental Work

I. Method of Isolation.

The given cultures were first streaked onto Saboraud's* agar slants,

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* Saboraud's agar: Peptone 10 gm., Dextrose 40 gm., Agar 15 gm., Water 1000 cc.

and incubated at 37° C. for 48 hours. This temperature was chosen because the growth of the Blue Stain fungi is definitely inhibuted; and after several such transfers, no fungus could be found.

A few of the cultures, however, were still contaminated with bacterial forms. To eliminate these latter, several repeated transfers of the cultures in dextrose broth at a pH 2.8 - 3.0 were made. This high acidity kept down the bacterial growth, while permitting the yeasts to grow quite well. After no further bacteria could be detected, the cultures were streaked out on Saboraud's agar plates, and individual colonies picked, and transferred to slant cultures. Next, these slants were plated out, by the dilution method, in triplicate, and three or more sub-isolations made. Except for cultures, 4, 9, and 12, the triplicates were apparently identical. The differences in these three cultures are given in the table.

II. Morphologic Characters

Microscopic examination of young cultures on Saboraud's agar showed all to exhibit true yeast cell forms, with budding processes. In addition, certain cultures showed pseudo-mycelium, while others possessed true mycelium. It is interesting to note that all those cultures from Metaline Falls have shown the true mycelium formation, while only 9 c from Gaspé showed true mycelium.

In order to study sporulation, the cultures were grown in carrot extract agar*, and carrot slice slants. To date, only cultures 2 and 5 have given positive spore formation, these having produced the "hat-shaped" spores, typical of the genus Willia Hansen.

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* Carrot extract agar: Extract of 1000 gm. carrots; $CaSO_4$, 10 gm; Agar, 20 gm.; Water 300 cc.

In studying the type of cell produced, and sporulation, wet mounts in aqueous Erythrosin were employed, and examined under 1000 X magnification.

III. Cultural Characters.

Growths in agar slant, deep agar stab, giant colony, and dextrose broth cultures were used in determining the cultural characters of the organisms in question. The four cultures from Wyoming, which showed true mycelium under the microscope, again exhibited this trait microscopically on the agar media. In the stab cultures, radiating mycelia were sent out from the line of inoculation; and on the surface growths, mycelial penetrations down into the agar. This peculiarity seems to be related to a reduced oxygen supply - which is in accordance with reports of other investigators relative to the Monilia. The photograph of culture 11 shows this typical "fir-tree", or villous, type of growth given by the aforementioned four cultures, as compared with culture 1. An interesting contrast also appears in the photos of the giant colonies of the same two cultures.

IV. Physiologic Characters.

The following points in physiology of the twelve isolations have been determined.

1. Oxygen relations. The fact that growth occurs in the deeper parts of the stab cultures, where there is only a limited supply of atmospheric oxygen, shows ability to grow in semi-anaerobic culture. Some other work, not yet advanced anough to be here reported, points to the fact that these cultures can grow under very anaerobic conditions.

2. Chromogenesis. No culture has ever shown any pigment production.

3. Diastatic Action. The cultures were all grown on 2 per cent starch agar. After four days, when there had been good growth, the plates were flooded with an iodine solution, to test for clearing due to starch digestion. The results were all negative. A few duplicates, however, held for 10 days, and then flooded, showed positive starch digestion. This has necessitated a repetition of the experiment, the plates to be held for a longer period before testing. These latter data are not yet available.

4. Cellulose Fermentation. Results to date are that cellulose is not digested. But, a longer period of observation is required before digestion can be definitely decided, positively or negatively.

5. Milk. Inoculation into litmus milk produced no apparent change, even after prolonged incubation.

6. Fermentation of Sugars. Yeast-water broth, to which had been added 1 per cent of the sugar being studied, was used in standard fermentation tubes. Fermentation is classed as positive if gas is produced, otherwise negative.

7. Temperature Relations. Growth was very slow at 4°C., and at 37°C.; these are probably quite close to the maximum temperature for growth. The optimum temperature is about 28°-30°C.

Discussion of Results.

From the data gathered, the cultures of the organisms seem to be, in some cases true yeasts, and in others some closely related forms, as Monilia. The Monilia (Gmelin), described by Guilliermond, describes these latter more accurately than the genus Monilia (Persoon). The two cultures producing hat-shaped spores probably are Willia (Hansen); however, none of the cultures can at present be positively identified. It is necessary to try

several more methods of inducing spore-formation for the remaining cultures, before definitely negative data can be given.

In the same way, the starch digestion and cellulose fermentation experiments are still in progress.

Suggested Future Work.

1. In the case of a few (1 or 2) cultures, it may prove advisable to isolate single cell cultures; as the data indicates a possibility of mixed cultures, not separable by ordinary plating methods.

2. It is intended that the physiology of the Blue Stain fungi be investigated. There seems to be only the data of Münch on this phase.* Also, the effect on starches, sugars, cellulose, etc., of these two organisms grown in symbiosis might yield some interesting data. Since the two forms, the yeast and fungus, are so closely associated in the beetle-infested trees, some mutually beneficial relationship must exist. The fermentation products of these organisms might also be of value, as some of them might be of a toxic nature. Also, some of the cultures of the yeast organism appear to be Monilia; and these, as a group, are very often pathogenic.

3. It has been said that the yeasts are always present in the intestinal tract of the Dendroctonus beetle. (Noted in report, from work in California, of the Forestry Department). This phase of the problem might also be of interest, although it is probably beyond the scope of the present problem.

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*"Die Blaufaule des Nadelholzes", Ernst Munch, pp. 65-69, 1907.

Table I

Morphology and Cultural Characters

	Culture number											
	1	2	3	4	5	6	7	8	9a	9c	10	11	12
Morphology	Y	Y	Y+M	Y	Y	Y+PM	Y+PM	Y+PM	Y	Y+M	Y+M	Y+M	Y+M
Stab culture	E	E	V	E	E	E	E	E	E	V	V	V	V
Slant culture	S	S	S+M	S	S	S	S	S	S	S+M	S+M	S+M	S+M
Broth culture	S	S	S	S	S	S	S	S	S	S	S	S	S+P
Source of culture	G.C	G.C.	W	G.C.	G.C.	G.C.	G.C.	G.C.	G.C.	G.C.	W	W	W

Code

Morphology

Y = Budding yeast cells
PM = Pseudo-mycelium
M = True mycelium

Slant culture

S = Surface growth only
S+M = Surface growth plus mycelia growth into agar

Stab culture

E = Echinulate
V = Villous (Fir tree)

Broth

S = Sediment
P = Pellicle formation (surface growth)

Source of culture

G.C. = Gaspe, Canada
W = Metaline Falls, Wash.

Table II

Physiologic Characters

	Culture number													
	1	2	3	4a	4b	5	6	7	8	9a	9c	10	11	12a	12b
Oxygen relation	Aerobic and semi-anaerobic														
Pigment production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch digestion	D	+	D	D	D	+	D	D	D	D	D	D	D	D	D
Cellulose digestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Action in milk	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sugar fermentation (Dextrose	+	-	±	-	+	+	-	+	+	+	+	+	+	+	-
Sugar fermentation (Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sugar fermentation (Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sugar fermentation (Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code

Starch digestion

D = Still doubtful

Sugar fermentation

+ = Gas
- = No gas